## Colony PCR

For confirmation of gene exchange clones, colony PCR is performed with respective verification primers. Clones for testing are prepared by boiling each clone in 50 µL TE-Buffer at 99°C for 10 min. A backup patching plate is prepared with the colonies patched on and incubated over the day at the appropriate incubation temperature. The colony PCR is performed with the comparatively cheap Taq DNA Polymerase, since it is only an analytic step. Each colony PCR is performed with 19 µL Taq DNA polymerase Master Mix plus 1 µL boiled clones.

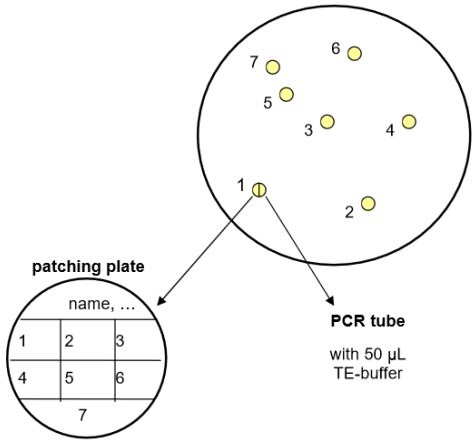


**Table 5: composition of reaction mixture for Taq DNA polymerase Master Mix**

| Component | Concentration in reaction mixture | Volume in 20 µL reaction mixture |
|---|---|---|
| **Nuclease free Water** | - | 13.7 |
| **10x Dream Taq Polymerase Buffer** | 1x | 2 |
| **25 mM $MgCl_2$** | | 1.2 |
| **10 mM dNTPs** | 200 µM each | 0.5 |
| **Primer Forward** | 0.5 µM | 1 |
| **Primer Reverse** | 0.5 µM | 1 |
| **Boiled colonies (Template DNA)** | - | 1 |
| **Taq DNA Polymerase** | | 0.6 |

**Table 6 : PCR program for Taq polymerase reaction**

| Step | Temperature / °C | Time / min | cycles |
|---|---|---|---|
| **Initial denaturation** | 95 | 3:00 | 1 |
| **Denaturation** | 95 | 0:30 | 30 |
| **Annealing** | 60 | 0:30 | 30 |
| **Extension** | 72 | x (60 sec / 1kb) | 30 |
| **End elongation** | 72 | 10:00 | 1 |
| **Hold** | 4 | - | - |

4 µL of 6x Loading Buffer is added to each reaction tube to obtain a final concentration of 1x. For analysis of PCR product or gel extraction, 1% agarose gel with 0.5 µL EtBr is prepared with appropriate number of loading pockets. 8-10 µL of PRC product (for analysis only; all sample for gelextraction) is loaded to one pocket for each sample. 5 mL of DNA ladder is loaded in one pocket of a documentation gel. Connect loaded gel to circuit by wiring up with +ve pole at bottom of gel chamber and –ve pole at top of gel chamber. Gel is run for ca. 40 min at 90 mV.